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Paper:

Disposable Inkjet Mechanism for Microdroplet Dispensing

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We succeeded in dispensing microdroplets using a disposable inkjet. The novelty of our device lies in the following points: (1) We used a glass-plate-bonded polydimethylsiloxane (PDMS) membrane to fabricate a leaf spring whose spring coefficient is 14 times higher than that of conventional PDMS membranes. This enabled droplets to be dispensed continuously up to 10 Hz when the membrane vibrated through the use of a multilayer piezoelectric actuator attached to the disposable PDMS chip. (2) The nozzle for droplet dispensing was fabricated from thick photoresist (SU-8) to obtain a hydrophobic surface preventing the production of undesired satellite droplets, yielding a droplet dispensing accuracy of $\pm$16.2 μm. Droplets produced by the disposable nozzle, which was 100 μm in diameter, ranged from 95-105 μm at an applied voltage of 105 V.

Keywords: inkjet, μTAS, microdevice, microfluidics

1. Introduction

Advances in microfluidics and labs-on-a-chip in biomedical applications have progressed with the automation of biomanipulation by an external magnetic or electrical field using a disposable PDMS chip. Numerous PDMS biochips with diverse functions have been developed [1–5], including cell manipulation in Embryonic Stem (ES) cell and cloning differentiation experiments [6–11]. Little research has been done, however, on retrieving manipulated cells systematically from chips. Cells must be individually distributed when all processing is completed on a chip and placed in an incubation atmosphere, often done manually or using a cell sorter.

Flow-cytometry-based cell sorters sort cells in a continuous cell-laden flow and collect sorted cells in cell suspensions [12–14]. Cell sorting is generally either electrical by cell charging or mechanical by moving receiving dishes after sensing cells by laser illumination as cells pass, with the laser pulse controlled so that single cells are placed in single droplets by thrusting the diluted cell suspension into an air phase. This usually large, expensive system makes it difficult to situate many functions before the ejection area, underscoring the need for a low-cost cell sorter-on-a-chip that ejects cells in culture wells.

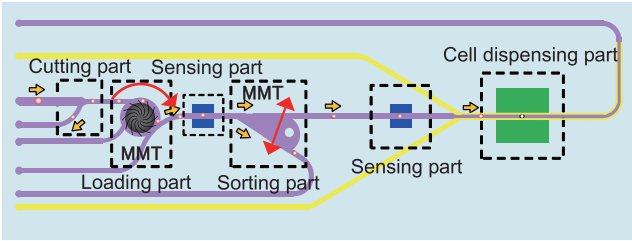

Fig. 1. On-chip cell manipulation and cell-laden droplet dispensing concept.

Cells are preferably injected immediately after operation is completed on the chip so automating the chip-world interface supplying cells from the biochip to the incubation atmosphere is highly desirable.

We have studied automating egg cell cloning. In previous research we reported loading cells one by one and sorting them automatically using an image sensor. We are now targeting an all-in-one approach from loading and sorting to individual cell dispensing, as shown in **Fig. 1**. Individual cells loaded on the chip by a rotary magnetically driven microtool (MMT) [9, 10] pass a sensor [9] and are sorted by lateral MMT movement blocking one of the microchannels. After sorting, cell-laden droplets are dispensed from the chip exit at a speed controlled by dispensing timing through sensing.

We applied the inkjet to the biochip chip-world interface, but using biological materials such as cells tends to spread contamination. The conventional inkjet consists of a solution tank, nozzle that is difficult to modularize to making cleaning easy and disposable [15–19].

We propose automating on-chip particle retrieval by spotting on a microarray using a novel disposable inkjet that avoids contamination risk [20]. Its advantages include a reusable drive and disposable biochip. Biomanipulation using inkjet technology is not new, of course, but has not used a disposable structure, making them expensive. This paper consists of five sections – Section 1 as

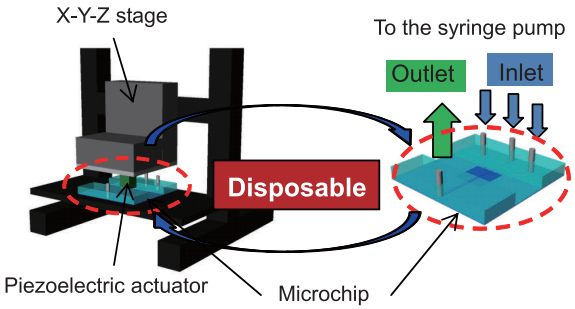

Fig. 2. Disposable microdroplet dispenser.

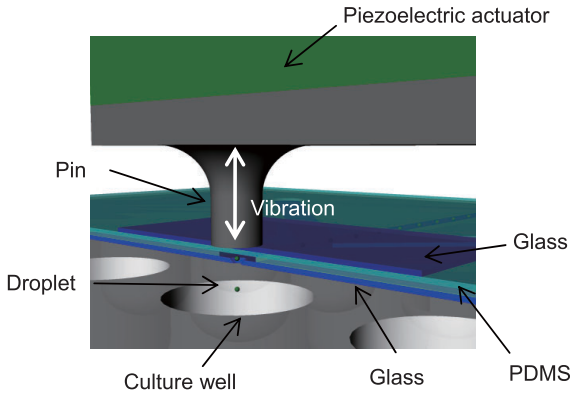

Fig. 3. Droplet generation.

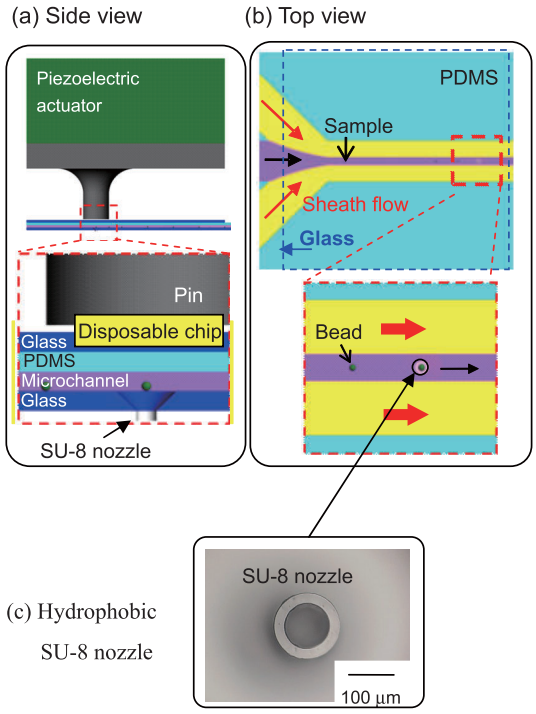

Fig. 4. Droplet generation and hydrophobic SU-8 nozzle fabricated to solve leakage and satellite generation.

Table 1. Piezoelectric actuator properties.

Generative force [N]	3500
Electrostatic capacitance [μF]	5.4
Maximum displacement [μm] (Maximum applied voltage is 150 V)	18.4 $\pm$ 3.5
Resonance frequency [kHz]	69

the introduction, Section 2 presenting the inkjet principle, Section 3 detailing analysis and fabrication, Section 4 describing inkjet operation and evaluation and bead ejection experiments, and conclusions summarized in Section 5.

2. Inkjet Principle

The disposable on-chip inkjet [20] we propose prevents contamination because the biochip microfluid network, shown in **Fig. 2**, is disposable. The independent drive consists of a multilayer piezoelectric actuator (AE1010D16F, Nectokin) and an aluminum pin. The disposable biochip has a glass-plate-bonded PDMS membrane and SU-8 nozzle. A glass plate between the actuator and PDMS membrane increases rigidity. Controlling the syringe pump flow to produce a laminar sheath flow [21–23], we supplied solutions to the nozzle leakage-free. The hydrophobic SU-8 nozzle prevents undesirable satellite droplets around the main droplet as shown in **Figs. 3** and **4**. Droplets are generated because the piezoelectric actuator vibrates the membrane in the upper part of nozzle and ejected droplets are supplied to the culture well.

3. Dispensing Design and Fabrication

The inkjet consists of a multilayer piezoelectric actuator fixed on a precision XYZ stage whose resolution is 1 μm, positioning accuracy 20 μm, replication accuracy 20 μm, and range of movement $\pm$25 mm. The pin fixed on the piezoelectric actuator is on the upper PDMS chip roof. **Table 1** lists piezoelectric actuator parameters used in experiments.

The disposable biochip is fabricated using sandblasting, photolithography, and molding as shown in **Fig. 3**, make it suitable for low-cost mass production. Sandblasting is used to make holes in substrates. That using patterned film photoresist is especially high-precision microfabrication.

The sandblaster (MB1-ML, SintoBrator, Ltd.) used in experiments has the following advantages:

(1) High process accuracy of $\pm$5 μm

(2) High process rate

(3) Complex shape processing

(4) Minimum glass cracks and chipping

The sandblasted nozzle is tapered for droplet generation.

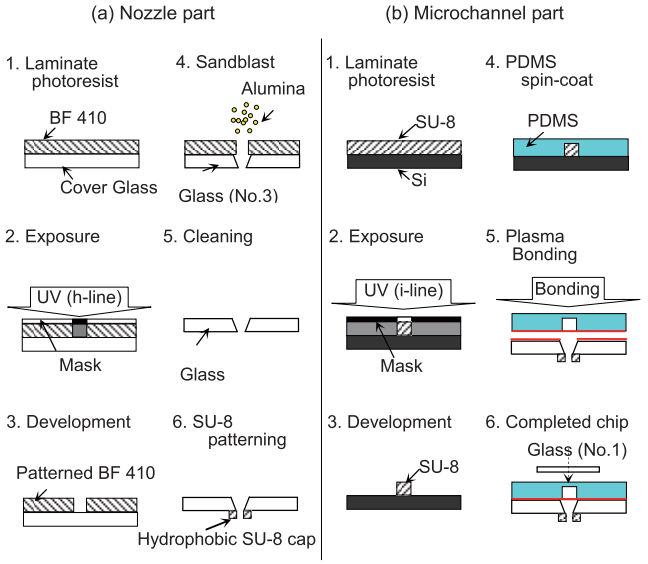

Fig. 5. Disposable biochip fabrication. (a) Nozzle, (b) microchannel.

A glass plate ($t = 300$ μm) is coated with negative photoresist BF410 that is exposed under ultraviolet (UV) light (h-line) at a 200 mJ/cm^2 exposure dose and developed for 10 min with 0.3% Na_2CO_3 developer. The developed substrate is rinsed in deionized (DI) water for 30 s, and the tapered hole is sandblasted for the nozzle on the glass substrate. To improve water-repellence, an SU-8 nozzle 100 μm in diameter ($t = 50$ μm) is fabricated under a glass hole 100 μm in diameter as shown in **Fig. 5**.

To fabricate the microchannel, a silicon substrate is coated with negative photoresist SU-8 ($t = 100$ μm), which is then exposed under UV light (i-line) at a 600 mJ/cm^2 exposure dose, then the microchannel pattern is fabricated. PDMS is then spin-coated at 1,000 rpm for 20 s on a patterned substrate to produce a thin PDMS membrane with a microchannel pattern. The PDMS sheet is cured by heating in an oven at 90°C.

The nozzle and microchannel are bonded, and the disposable biochip is fabricated as shown in **Fig. 5**.

Biocompatible PDMS is widely used as a biochip material. The fabricated system injects cell-laden droplets.

We found that membrane rigidity is important in continuous droplet generation. We propose using a rigid plate's large elastic restoration force to recover deformed membranes and dispense droplet continuously. We tested glass and phosphor bronze as rigid materials. Glass is easy to install, inexpensive, and biocompatible. Phosphor bronze is rigid and often used for springs.

Figure 6 shows Finite Element Method (FEM) analysis results for Mises stress around a deformed membrane with and without glass plate reinforcement. FEM analysis used the COMSOL 3.5 structural mechanics module. The pin was displaced 10 μm in both cases. Force needed to deform the membrane was 8.8 N with the glass plate but only 0.65 N without the glass plate. Analysis assumed 2-dimensional (2D) analysis because displacement at the glass center and periphery is negligible, within 2%.

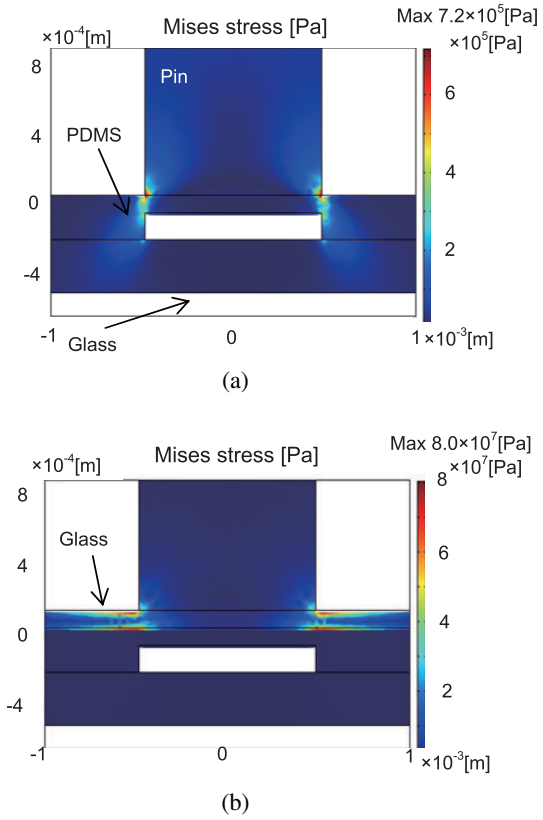

Fig. 6. FEM analysis of Mises stress. Pin displacement was 10 μm in both cases. (a) Membrane without glass, (b) membrane with glass.

Table 2. Glass and PDMS Properties.

	Young's modulus [Pa]	Poisson Ratio	Density [kg/m^3]
PDMS	2×10^6	0.30	1110
Glass	70×10^9	0.17	2200
Phosphor bronze	110×10^9	0.33	8900

Table 2 lists PDMS, glass, and phosphor bronze property, showing that elastic restoration is large with the glass plate and stress on the membrane is equally distributed along the glass using the glass plate, giving the effect of a leaf spring and increasing elastic restoration. Spring constants of glass-bonded and conventional PDMS membranes were compared by

$$F = kx \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

The glass-bonded PDMS membrane spring coefficient was 14 times that of the conventional PDMS membrane, and the glass-bonded PDMS membrane acted as a leaf spring.

Force required to deform the phosphor bronze membrane was 9.8 N, and the spring coefficient was 15 times that of the conventional PDMS membrane, but we

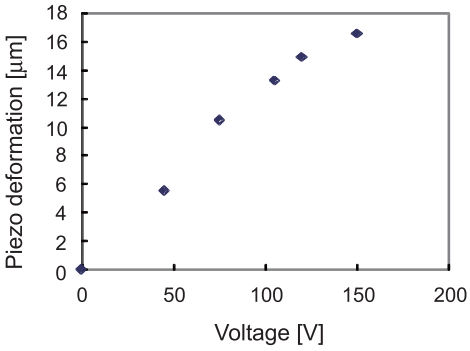

Fig. 7. Piezoelectric actuator performance.

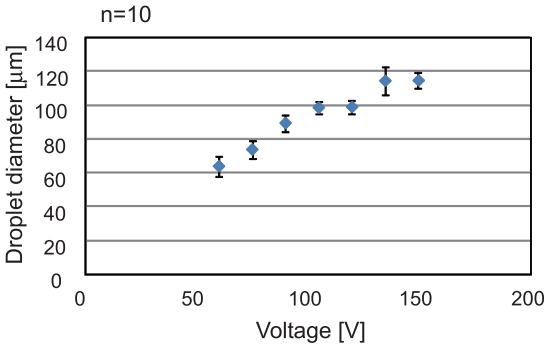

Fig. 9. Injected droplet size as a function of applied voltage.

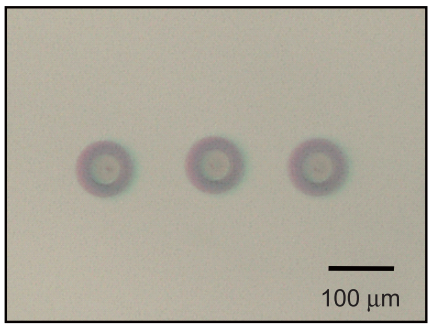

Fig. 8. Microdroplets dispensed in oil.

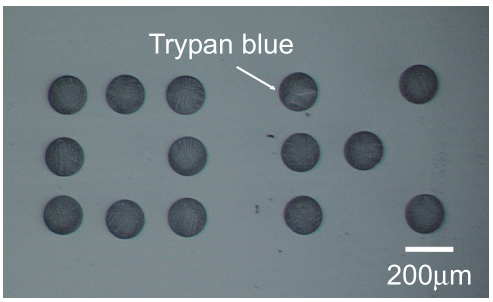

Fig. 10. Automated spotting on a microarray.

used glass as the membrane material because it is clear, whereas phosphor bronze is opaque. With the glass plate transparent, it is easy to adjust pin positioning, and the spring coefficient of glass is relatively large.

4. Results and Discussion

4.1. Droplet Generation Evaluation

To demonstrate droplet generation in experiments on a chip, we used an applied voltage of 15-150 V, a pulse width of 1.5 ms, and a nozzle 100 μm in diameter. Before experiments, the piezoelectric actuator was positioned so that the tip touched the glass substrate, as observed by an inverted microscope, preventing any risk of glass cracking or damage in initial setup. By controlling syringe pump flow at 1 ml/h per syringe center and 2 ml/h for two syringes producing laminar sheath flow and 5 ml/h for extraction from the exit, we prevented the leaks of solutions from the hydrophobic SU-8 nozzle, which in turn prevents undesired satellite droplets around the main droplet. The piezoelectric actuator is displaced as shown in **Fig. 7**. The displacement was measured using a microscope. Size of the DI water droplets dispensed to the oil was measured. **Fig. 8** shows droplets dispensed into oil, confirming that droplet size increased monotonically with increasing applied piezoelectric actuator voltage. Oil surface tension greatly exceeds that of water, making water spherical in oil. When the edge of the sphere is focused, the inner part becomes defocused due to focal depth limitation, so we measured droplet size by focusing on the outer edge. Droplets produced by the disposable nozzle, which is 100 μm in diameter, ranged from 95 to 105 μm at an applied voltage of 105 V for the piezoelectric actuator as shown in **Fig. 9**. Data was recorded for voltage exceeding 50 V, since no droplets were generated at a lower voltage.

4.2. Frequency Response Evaluation

We evaluated droplet generation frequency response for the current setup to achieve high throughput, which requires high-frequency droplet generation. The maximum frequency for the current setup was 10 Hz. We observed droplet generation using an inverted microscope. Frequency was increased by maximum frequency generating droplets without damaging glass, which was broken at higher frequencies. Getting better high-speed injection requires optimizing parameters such as membrane material, and pin shape.

4.3. Inkjet Droplet Patterning

Droplet dispensing accuracy was confirmed in performance assessment. Trypan blue (0.4%) droplet patterning dispensed onto a glass substrate was successfully done at equal intervals and dot arrays as shown in **Fig. 10**. Droplets averaged 177 μm. We measured spotted droplet diameter and succeeded in individual satellite-free droplet patterning. Accuracy in droplet dispensing positioning was $\pm$16.2 μm, compared to the 5 mm distance between

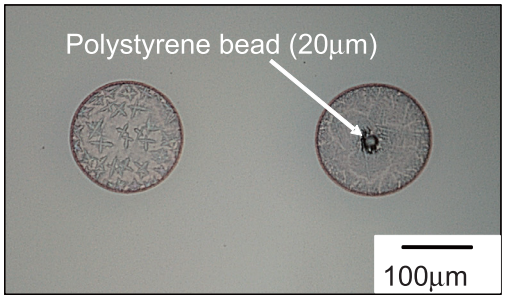

Fig. 11. Polystyrene bead dispensing by inkjet.

the nozzle and plate. Different patterns are expected to be drawn by controlling the stage, and inkjet use will enable reagent or cell droplets to be dispensed well to culture wells.

4.4. Inkjet Microbead Dispensing

We demonstrated the ejection of polystyrene beads 20 μm in diameter in initial experiments on cell-laden droplet injection. Microbeads were mixed with trypan blue and were supplied in the biochip. We used the laminar sheath flow to focus flowing particle positioning at the center of the microchannel, and 20 μm polystyrene-bead-laden droplets were successfully injected as shown in **Fig. 11**. In projected study, injection timing will be controlled by a capacitance sensor. Microelectrodes are patterned on the microchannel substrate to detect changes in capacitance when the cell flows off electrodes. A cell-laden droplet is injected when the trigger signal is sent to the piezoelectric actuator. We also plan to evaluate and optimize fluid timing and control and system design.

5. Conclusions

We have succeeded in dispensing particle-laden microdroplets using a disposable on-chip inkjet under experimental conditions of 15-150 V applied voltage, 1-10 Hz frequency, 1.5 ms pulse width, and 100 μm nozzle diameter. Droplets produced by the disposable nozzle ranged from 95 to 105 μm at an applied voltage of 105 V. Accuracy in droplet positioning was $\pm$16.2 μm. We also tested a maximum frequency of 10 Hz, which may be improved in future by optimizing parameters.

The glass-bonded PDMS membrane spring coefficient was 14 times that of the conventional PDMS membrane. Droplets were generated continuously because membrane rigidity increased and restoration was improved. Other materials can, of course be considered in improving the membrane spring coefficient. Glass is easy to install, inexpensive, and biocompatible and, because it is transparent, it is easy to adjust pin positioning. The droplet dispensing nozzle was fabricated using SU-8 to obtain a hydrophobic surface preventing undesired satellite droplets. By controlling syringe pump flow at 1 ml/h per syringe (center) and 2 ml/h for two syringes to produce a laminar sheath flow and 5 ml/h extraction from the exit, we prevented the leaks of solutions from the nozzle.

We thus plan to expand our system to automate cell-laden droplet spotting on a microarray to culture cells efficiently, eventually developing a desktop plant that achieves a series of automated operations, such as cell loading, separation, dispensing, and culture.

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1994- Assistant Professor, Nagoya University
1998- Associate Professor, Nagoya University
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Main Works:
• F. Arai, C. Ng, H. Maruyama, A. Ichikawa, H. El-Shimy, and T. Fukuda, "On Chip Single-Cell Separation and Immobilization Using Optical Tweezers and Thermo Sensitive Hydrogel," Lab on a chip, Vol.5 No.12, pp. 1399-1403, 2005.
• T. Fukuda, F. Arai, and L. Dong, "Assembly of Nanodevices with Carbon Nanotubes through Nanorobotic Manipulations," Proc. of the IEEE, Vol.91 No.11, pp. 1803-1818, 2003.

Membership in Academic Societies:
• The Japan Society of Mechanical Engineers (JSME)
• The Robotics Society of Japan (RSJ)
• IEEE, Robotics and Automation Society